The State of Insulin in the Blood*

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In discussing the state of insulin in the blood, I should like to review briefly the regulation of insulin secretion and what is known about its manner of synthesis and release. Since some of the insulin in the blood may be in an ineffective or inactive state, I shall refer to active insulin as *effective* insulin.

METABOLITE STIMULATORS

The stimulus acting on the pancreas that controls the level of effective insulin in the blood is probably related to more than one metabolite. For many years glucose was considered the only substance capable of insulin stimulation, but recently it has been shown that keto-acids (Mebane and Madison, 1962) and certain amino acids such as leucine and arginine (Floyd et al., 1965; Merimee et al., 1965) can also stimulate insulin release. There is need to learn whether this has physiological significance before assigning it a specific role in the checks and balances of human metabolism. Intravenous studies using arginine will be followed by oral feedings of appropriate animal proteins to determine whether or not there will be an increase in the level of serum insulin without a rise in blood sugar.

SENSING-RELEASING MECHANISMS

The manner in which glucose or other metabolites bring about the secretion of insulin is unknown. It is reasonable to postulate that a sensing device must be present that recognizes the amount of the specific stimulator present and informs the pancreas that insulin is needed. This immediately raises the possibility that a pathologic state could exist wherein a faulty sensing device would fail to inform the pancreas of the need, and insulin would not be secreted despite the presence of normal quantities of insulin in the pancreas. Studies with the sulfonylurea drugs may perhaps be interpreted as bearing on this question. It is clear that insulin can exist within the islet cells quite unaware of the hyperglycemic blood flowing past and that sulfonylurea drugs can bring about the release of this insulin. These drugs may activate a sensing device. It is equally possible that they may activate a releasing mechanism for we cannot separate these two postulated functions clinically. In individuals with adequate pancreatic stores of insulin that are not responsive to elevated levels of glucose, the sulfonylurea drugs bring about a more normal response to the stimuli. There is increasing evidence that there is carbohydrate intolerance that advances with age (Andres, 1966, in press) and again it seems likely that this relative insensitivity comes about through a failure either of the sensing device

* The schema presented here is the result of much discussion involving all members of the endocrine division at the Johns Hopkins Hospital. Dr. Kenneth Zierler, Dr. David Rabinowitz, and Dr. Thomas Merimee are especially cited in the formulation of these concepts.

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or releasing mechanism. Perhaps other conditions which alter insulin release, such as the glucose intolerance following fasting, may relate to these mechanisms.

SYNTHESIS AND STORAGE

Although we can say little about the mechanisms of release of insulin, we are indebted to the beautiful studies by Lacy (1961) with the electron microscope for an exposition of the migration of the insulin granule across the beta-cell to the cell surface. On reaching the cell surface, the envelope of the beta granule appears to coalesce with the surface membrane where upon the beta granule is discharged into the extracellular space. At this point, unfortunately, the resolution of the electron microscope does not allow further physical view of the discharged granule. After discharge of the previously stored insulin, empty sacks are again seen in the beta cell; these are frequently surrounded in part by a protein believed to be ribonuclear protein. The ribonuclear protein granules clustered about the sack slowly disappear as the amorphous material fills the empty sack, and it is easy to presume that we have witnessed the synthesis of insulin on a template. The amorphous material in the sack is next seen to condense into a storage form that is typical of the beta granules previously discharged. The discharge of these granules in response to known stimuli such as glucose and sulfonylurea drugs adds further to the circumstantial evidence that we are in fact witnessing the synthesis and release of insulin.

Randle and his associates (*see* Coore and Randle, 1964) have been able to show that insulin can be released from pancreatic slices in vitro by the addition of glucose to the medium. In this system, epinephrine inhibits the release of insulin by both glucose and the sulfonylureas. Perhaps the most important observation has been that he has been able to prevent the release of insulin from the pancreas by the addition of manoheptulose when glucose is added to the medium, but manoheptulose does not prevent the release of insulin under the stimulus of the sulfonylureas. This is the first time that anyone has been able to separate the release mechanisms associated with glucose from those related to the sulfonylureas and parenthetically this suggests that the release mechanism rather than the sensing device is the site of action of the sulfonylureas in human subjects.

CIRCULATING INSULIN

In considering the state of insulin in the blood, we must deal with certain direct questions: What is its form? How is it transported? And what is the material being measured which we call "insulin?" These are fundamental questions, but our answers must be incomplete.

From the work of Sanger (1960) we have the precise structure of the insulin molecule, and the molecular weight is known to be approximately 6,000. It has not been possible to be certain of the form taken by circulating insulin. It probably circulates as a double molecule or dimer with a molecular weight of 12,000, but it may circulate in aggregates of three or four molecules (Prout, 1963). There is some evidence that the insulin may be transported in the blood attached to a carrier protein, but this cannot be definitely proven at this time.

Insulin is measured in biologic fluids on the basis of its biologic effect or its immunologic properties. In general, there are two types of biological assay, one measuring the effect of glucose uptake or glycogen deposition in a rat hemi-diaphragm and the other measuring the incorporation of C^{14} from labelled glucose into glycogen or CO_2 by the rat epididymal fat pad. Both of these tests measure a net biological effect which may be altered as a result of several modifying factors either potentiators or inhibitors. For this reason, the activity thus measured is referred to as the "insulin-like activity" or ILA. The immunoassay of Berson and Yalow (1960) identifies immunologically active insulin quite specifically and with considerable sensitivity; it is, of course, able to tell us nothing about the biological activity of the thing that is measured. About these two types of assay have grown up two schools of thought which divide the investigators interested in this field.

Faced with this dilemma, it is of some comfort to consider the number of ways in which the ILA as measured by the diaphragm and the immunoassay give parallel results (table 1). By both tests the blood insulin is low in the fasting subjects but rises after glucose or after administration of the sulfonylureas. By both tests the blood insulin is high in patients with insulin secreting islet cell tumors. In obese patients both assay techniques reveal increased insulin levels. The levels may be high by both assay methods in certain adult diabetes, and most importantly immunologically active insulin and ILA as tested by the rat hemi-diaphragms are both

TABLE 1
Similarities in the Assay of Insulin in Whole Serum by the Rat Diaphragm and by the Immunoassay

1) Low insulin levels in blood of fasting patients
2) Rise of insulin after glucose administration
3) Rise of insulin after sulfonylurea administration
4) High levels of insulin in hyperinsulinism
5) Elevated levels of insulin in some obese subjects
6) Suppression of insulin in vitro and in vivo by specific insulin antibodies
7) Disappearance of insulin after pancreatectomy
8) Absence of insulin in serum of patients in diabetic keto-acidosis

suppressed by anti-insulin antibodies either in vitro or in vivo. Finally, the insulin measured by either method disappears when the pancreas has been removed. In these respects both assays would appear to be measuring the same material.

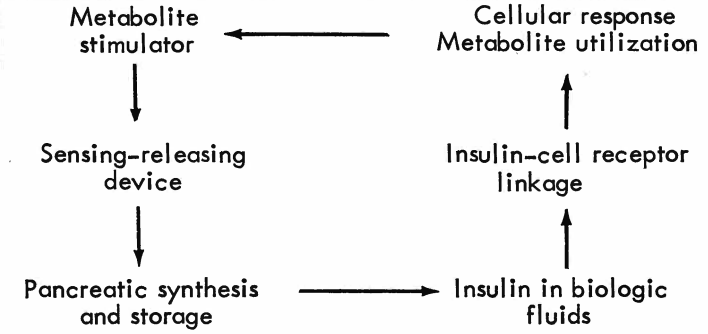
The observation that insulin-like activity measured by the fat pad was several fold that measured by the diaphragm or the immunoassay turned attention to the question of whether or not a circulating form of insulin existed which had lost, or perhaps had never had, immunologic identity. By means of a cation exchange resin column, Antoniades and his associates (1964) separated a form of insulin-like activity that could be measured initially by the fat pad but not by the diaphragm or immunoassay. This was called "bound" insulin as opposed to the "free" form. Others have found insulin-like activity that is "atypical" (Samaan et al., 1963) or "non-suppressible" (Froesch et al., 1963) because the activity unlike that of crystalline insulin is not inhibited by anti-insulin antisera added to the test medium. We can conveniently consider together these various types of "bound," "complexed," "non-suppressible," or "typical" insulins and contrast them to insulin that is measurable by the other methods (table 2).

One difficult problem confronting the proponents of atypical insulin in that some "atypical" insulin seems to persist in the blood for considerable periods of time after pancreatectomy (Goldberg and Egdahl, 1961). Before attempting to give clinical relevance to ILA of the "atypical" type, a second observation requires physiologic explanation. We are asked, for example, to believe that ILA measured only by the fat pad is circulating in excess quantities in patients with decompensated diabetes in whom the principle evidence of decompensation is failure of insulin to act normally upon fat synthesis (Steinke et al., 1961). These criti-

TABLE 2
Differences Between "Typical" and "Atypical" insulin

	"Typical"	"Atypical"
Assay	Measured by diaphragm fat-pad and immunoassay	Measured by fat-pad
Effect of antiserum	Inhibited	Not inhibited
Effect of pancreatectomy	Disappears	Remains
Origin	Pancreas	Unknown
Keto-acidosis	Absent	Present in large quantity
Other unresolved questions	Biologic activity of insulin measured immunologically is not known	Cannot be converted reproducibly to typical form

TABLE 3
The Feed-back Mechanism of Insulin Synthesis and Release



cisms have lead many to deny that "atypical" insulin has any real relationship to true pancreatic insulin. Despite these difficulties, it is theoretically possible that some form of insulin may exist which is different from the insulin measured immunologically and by its biologic effects on muscle and further study of the problem will be necessary.

A FEED-BACK MECHANISM

We can now picture the glucose insulin regulating mechanism as a feed-back system (table 3). A metabolic stimulator, usually glucose, is measured by a sensing device which tells the pancreatic cell to release and re-synthesize insulin in relation to metabolic need. After circulating insulin has arrived in the blood, it is picked up by a specific cell receptor and initiates a cellular response. The cellular response not only serves a metabolic need but also removes from the blood some of the metabolic stimulator that initiated the cycle. As a result of the fall in the metabolite, no further stimulation for insulin release is given, and the feed-back mechanism is satisfied. Various other factors can presumably inhibit or potentiate the different steps of this process.

In this framework the diabetes with which we have been most familiar in the past is associated with failure of insulin release from the pancreas. This is most typically exemplified as the pancreatic exhaustion of juvenile diabetes. It is also true of the pancreas that has been destroyed by pancreatitis, hemochromatosis, or removed by the surgeon's knife. It may be seen where pancreatic exhaustion results from metabolic stresses such as hyperthyroidism, multiple pregnancies, or long-standing obesity. In all cases the pancreas is no longer able to respond to demand, and clinical diabetes is seen. In true pancreatic exhaustion the sulfonylureas are, of course, ineffective.

We should also note that a state of functional low insulin output exists where there is either dulling of the sensing device or interference with insulin release; either of these mechanisms impairs the ability of the pancreas to deliver insulin on demand. In these instances, the sulfonylureas release insulin. Epinephrines and the thiazides inhibit release of insulin.

The new concept that must be brought into this scheme is the clinical situation in which metabolic abnormalities of the type seen in diabetes are present in spite of high circulating levels of insulin. A state of impaired carbohydrate intolerance in association with a high level of plasma insulin can be understood if the insulin present is ineffective. It is also possible to recognize the clinical syndrome in which normal high levels of insulin compete with antagonists such as growth hormone, acromegaly, or adrenal steroids. A similar though less well defined impairment of insulin is seen in association with obesity. Here again we may see impaired carbohydrate tolerance in the presence of high circulating levels of insulin (Karam et al., 1963). In this state it appears likely that the impairment to insulin function is located primarily at the level of cellular response. The same may be true in carbohydrate induced hypertriglyceridemia, a condition closely allied to diabetes but not necessarily true diabetes. There is some evidence that the lipoatrophic form of diabetes may be characterized by high levels of circulating insulin that are rendered ineffective by an insulin antagonist (Louis et al., 1965).

"Prediabetes" may take one of two forms. It may be a state in which high levels of insulin are put out to overcome peripheral impairment or circulating antagonists but in which carbohydrate intolerance cannot be demonstrated by ordinary techniques. This state is readily understood within the present framework. "Prediabetes" may also be present in a patient who shows

evidence of the "complications" of diabetes before carbohydrate tolerance is lost. There is some evidence that different tissues react to different levels of insulin and by presumption that levels of insulin adequate for the metabolism of some tissue would be ineffective for others. This may well be the reason why it is possible to see degenerative complications in the retina that is typical of diabetic retinopathy in some patients who have no carbohydrate intolerance. The same may be true of patients who develop vascular disease in the absence of alterations in carbohydrate utilization. Perhaps the future study will allow us to demonstrate that tissue sensitivity in such patients is the cause of the degenerative complications. There is abundant evidence that complications may be seen in the absence of carbohydrate impairment, and we can no longer accept the frequently stated belief that control of carbohydrate metabolism in diabetes will prevent all other forms of the "complications."

Much of the theme presented here can be verified, but much of it still remains speculative. It is probably possible to place all variants of the diabetic state somewhere within this cycle so that diabetes will eventually become better understood as a complex metabolic disorder involving all tissues and all metabolites rather than the simple derangement of carbohydrate utilization.

SUMMARY

Diabetes mellitus has been reviewed as a group of conditions with impaired function of one or more portions of a feed-back system involving the release and utilization of insulin. It is hoped that this may form a useful scheme by which we can study and understand a number of complex metabolic states which we must still collectively refer to as diabetes mellitus.

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